

Data Evaluation Report on the Acute Toxicity of Aminocyclopyrachlor acid technical to Algae (*Skeletonema costatum*)

PMRA Submission Number {.....}

EPA MRID Number 47560202

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	358148
	OECD Data Point	{.....}
	EPA MRID	47560202
	EPA Guideline	OPPTS 850.5400 (158.660)

Test material: Aminocyclopyrachlor acid technical **Purity:** 92.2%

Common name

Chemical name:

IUPAC 6-Amino-5-chloro-2-cyclopropylpyrimidine-4-carboxylic acid
 CAS name 6-Amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid
 6-Amino-5-chloro-2-cyclopropyl-pyrimidine-4-carboxylic acid
 CAS No. 858956-08-8
 Synonyms DPX-MAT28, Aminocyclopyrachlor

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Company Code {.....} [For PMRA]
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EPA PC Code None

Date Evaluation Completed: {dd-mm-yyyy}

CITATION: Porch, J.R., Kendall, T.Z., and H.O. Krueger. 2008. DPX-MAT28 Technical: A 96-Hour Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Unpublished study performed by Wildlife International, Ltd., Easton, Maryland. Laboratory Study No.: 112A-235. Study sponsored by E.I. du Pont de Nemours and Company, Wilmington, Delaware. Study completed May 7, 2008.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of



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factors related to the test methodology and results in determining the acceptability of the study.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the marine diatom (*Skeletonema costatum*) were exposed to Aminocyclopyrachlor acid technical at nominal concentrations of 0 (negative control), 7.5, 15, 30, 60, and 120 mg ai/L under static conditions. Mean-measured concentrations were <0.0281 (<LOD, control), 7.85, 15.5, 28.9, 60.0 and 120 mg ai/L.

Inhibitions for cell density ranged from -6.3 to 5.5% in the treatment groups as compared to the controls. Inhibition for biomass ranged from -11 to -0.4%. Growth rate inhibitions ranged from -1.8 to 1.5%. The most sensitive endpoint could not be determined due to extremely low inhibitions at every test level for every endpoint evaluated, resulting in an overall NOAEC and EC₅₀ value of 120 and >120 mg ai/L, respectively.

The study authors reported that cell morphology in the control and treatment groups was normal in size, shape, and color. There was no flocculation, aggregation, or adherence of cells to the test chambers in any group.

This toxicity study is scientifically sound and classified as acceptable. It satisfies the guideline requirement for a nonvascular aquatic plant toxicity study with the marine diatom, *Skeletonema costatum*.

Results Synopsis

Test Organism: *Skeletonema costatum*

Test Type (Flow-through, Static, Static Renewal): Static

Cell Density

EC₀₅: >120 mg ai/L 95% C.I.: N/A

EC₅₀: >120 mg ai/L 95% C.I.: N/A

NOAEC: 120 mg ai/L

Probit Slope: N/A

Biomass (Area Under the Growth Curve)

EC₀₅: >120 mg ai/L 95% C.I.: N/A

EC₅₀: >120 mg ai/L 95% C.I.: N/A

NOAEC: 120 mg ai/L

Probit Slope: N/A

Growth Rate

EC₀₅: >120 mg ai/L 95% C.I.: N/A

EC₅₀: >120 mg ai/L 95% C.I.: N/A

NOAEC: 120 mg ai/L

Probit Slope: N/A

Endpoint(s) Effected: None

I. MATERIALS AND METHODS

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GUIDELINE FOLLOWED: This study was conducted following OECD Guideline for Testing of Chemicals, 201: *Alga, Growth Inhibition Test*, and U.S. EPA Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Guideline 850.5400: *Algal Toxicity, Tiers I and II*. The following deviations from OPPTS 850.5400 were noted:

1. Total organic carbon, particulate matter, and chlorine concentrations of the well water used for the algal medium were not reported.
2. The highest test level had a pH of 7.4 at test initiation, which was lower than the minimum pH of 8.0 recommended by OPPTS guidelines for this species.
3. The physico-chemical properties of the test material were not reported.

These deviations do not affect the acceptability of this study.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP, and Quality Assurance statements were provided. This study was conducted in compliance with U.S. EPA FIFRA GLP standards (40 CFR Parts 160 and 792), that are consistent with OECD Principles of GLP (ENV/MC/CHEM(98)17), and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau, 1999).

A. MATERIALS:

1. Test material Aminocyclopyrachlor acid technical

Description: Solid

Lot No./Batch No. : DPX-MAT28-009 (Batch No.)

Purity: 92.2%

Stability of compound under test conditions: The day 0 measured concentrations yielded recoveries of 97% to 103% of nominal test concentrations, and day 4 measured concentrations yielded recoveries of 95% to 107% of nominal. Aminocyclopyrachlor acid technical was very stable under the test conditions.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Test material was stored at ambient temperature.

Physicochemical properties of Aminocyclopyrachlor acid technical.

Parameter	Values	Comments
Water solubility at 20EC	Not reported.	
Vapor pressure	Not reported.	
UV absorption	Not reported.	

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Parameter	Values	Comments
pKa	Not reported.	
Kow	Not reported.	

2. Test organism:

Name: Marine diatom, *Skeletonema costatum*
EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricornutum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: CCMP 1332
Source: In-house cultures originally obtained from Provasoli-Guillard National Center for the Culture of Marine Phytoplankton
Age of inoculum: At least 2 weeks prior to test initiation
Method of cultivation: Cultured and tested in saltwater algal medium

B. STUDY DESIGN:

1. Experimental Conditions

- a. Range-finding study A range-finding study was conducted, but no details were provided.
- b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Continuous	
Culturing media and conditions:	Saltwater medium	

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Parameter	Details	Remarks
		Criteria
(same as test or not)	Could not be determined.	<i>EPA recommends two week acclimation period.</i>
Health: (any mortality observed)	Algal cells appeared normal at test initiation.	<i>OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i>
<u>Test system</u> Static/static renewal	Static	<i>EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).</i>
Renewal rate for static renewal	N/A	
Incubation facility	Test vessels were placed on a mechanical shaker in an environmental chamber.	
Duration of the test	96 hours	<i>EPA requires: 96-120 hours OECD: 72 hours</i>
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Sterile Erlenmeyer flasks 250 mL 100 mL	<i>OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.</i>
<u>Details of growth medium name</u>		The saltwater medium was sterilized by filtration (0.22 µm) prior to use.

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Parameter	Details	Remarks
		Criteria
pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (for marine algae):	7.4-8.0 8.3-8.6 Yes. None 30 ppt	<p>OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.</p> <p>EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91 and D 3978-80 (reapproved 1987).</p>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
<u>Dilution water</u> source/type: pH: salinity (for marine algae): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Wildlife International well water Adjusted to 8.0 and 8.1 (2 lots of media were used in the study) N/A Water was purified Not reported. Not reported. Not detected to 34.9 mg/L Not detected. Not reported.	<p>EPA pH: <i>Skeletonema costatum</i> = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.</p> <p>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.</p>
Indicate how the test material is added to the medium (added directly or used stock solution)	A stock solution of 120 mg ai/L was prepared as the highest test concentration. This highest concentration stock solution was then serially diluted with algal medium.	

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Parameter	Details	Remarks
		Criteria
Aeration or agitation	Agitation, 100 rpm	
Initial cells density	7.7×10^4 cells/mL	<p>EPA requires an initial number of 3,000 - 10,000 cells/mL. For <i>Anabaena flos-aquae</i>, cell counts on day 2 are not required.</p> <p>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for <i>S. capricornutum</i> and <i>S. subspicatus</i>. When other species are used the biomass should be comparable.</p>
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 N/A 3	<p>EPA requires a negative and/or solvent control with 3 or more replicates per doses. <i>Navicula</i> sp. tests should be conducted with four replicates.</p> <p>OECD prefers three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.</p>
<u>Test concentrations</u>		

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Parameter	Details	Remarks
		Criteria
Nominal:	0 (negative control), 7.5, 15, 30, 60, and 120 mg ai/L	EPA requires at least 5 test concentrations in a geometric series with a ratio between 1.5 and 2.0.
Mean-measured:	<0.0281 (<LOD, control), 7.9, 15, 29, 60 and 120 mg ai/L	OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	Samples of the test solutions collected at 0 and 96 hours, quality control samples, and calibration standards prepared at 0 and 96 hours were analyzed using HPLC with UV (220 nm) detection.	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	19.4-20.6°C 16L:8D 4080-4880 lux Cool-white fluorescent lighting	EPA temperature: <i>Skeletonema</i> : 20°C, Others: 24-25°C; EPA photoperiod: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; EPA light: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%) OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.
<u>Reference chemical (if used)</u> name: concentrations:	N/A	
Other parameters, if any	None.	

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2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks
		Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	-cell density -area under the growth curve (biomass) -growth rate	<i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>
Measurement technique for cell density and other end points	Cell density was measured daily using an electronic particle counter. Growth rate and area under the growth curve were calculated using initial cell density and cell density at each time interval.	<i>EPA recommends the measurement technique of cell counts or chlorophyll a</i> <i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i>
Observation intervals	Every 24 hours.	<i>EPA and OECD: every 24 hours.</i>
Other observations, if any	N/A	
Indicate whether there was an exponential growth in the control	Yes, mean cell density in the negative control was 287.3×10^4 cells/mL at test termination.	<i>EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>
Were raw data included?	Yes.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 96 hours of exposure, cell density averaged 287.3×10^4 cells/mL in the negative control, yielding

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inhibitions of 1.8, 1.2, 3.9, 5.5, and -6.3% as compared to the negative control in the mean-measured 7.85, 15.5, 28.9, 60.0 and 120 mg ai/L treatment groups, respectively. The 96-hour NOAEC and EC₅₀ values based on cell density were 120 and >120 mg ai/L.

Growth rate averaged 0.0376 cells/mL/hour in the negative control, yielding inhibitions of 0.2, 0.1, 0.9, 1.5, and -1.8% as compared to the control. The 96-hour NOAEC and EC₅₀ values based on growth rate were 120 and >120 mg ai/L.

Biomass (area under the growth curve) averaged $11,541 \times 10^4$ in the negative control, yielding inhibitions of -1.7, -2.7, -0.4, -2.0, and -11% as compared to the negative control. The 96-hour NOAEC and EC₅₀ values based on biomass were 120 and >120 mg ai/L, respectively.

The study authors reported that cell morphology in the control and treatment groups was normal in size, shape, and color. There was no flocculation, aggregation, or adherence of cells to the test chambers in any group.

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Table 3: Effect of Aminocyclopyrachlor acid technical on algal growth (*Skeletonema costatum*)

Mean-Measured and (Nominal) Concentrations (mg ai/L)	Initial Cell density (x 10 ⁴ cells/mL)	Mean Cell density (x 10 ⁴ cells/mL) at				
		24 hours	48 hours	72 hours	96 hours	
					Cell count	% inhibition
Negative control	7.7	40.2	131.1	192.9	287.3	N/A
7.85 (7.5)	7.7	43.5	129.0	202.3	282.1	1.8
15.5 (15)	7.7	39.3	124.0	215.4	283.7	1.2
28.9 (30)	7.7	33.9	126.2	211.6	276.1	3.9
60.0 (60)	7.7	44.0	128.6	209.1	271.4	5.5
120 (120)	7.7	44.3	131.0	233.9	305.3	-6.3

N/A- Not Applicable

Table 4: Effect of Aminocyclopyrachlor acid technical on algal growth (*Skeletonema costatum*)

Mean-Measured and (Nominal) Concentrations (mg ai/L)	Initial Cell Density (x10 ⁴ cells/mL)	Mean Growth Rate (cells/mL/hour)		Mean Area Under the Growth Curve (Biomass) (x 10 ⁶)	
		0-96 Hours	% Inhibition	0-96 hours	% Inhibition
Negative control	7.7	0.0376	N/A	115.4	N/A
7.85 (7.5)	7.7	0.0375	0.2	117.3	-1.7
15.5 (15)	7.7	0.0376	0.1	118.5	-2.7
28.9 (30)	7.7	0.0373	0.9	115.9	-0.4
60.0 (60)	7.7	0.0371	1.5	117.7	-2.0
120 (120)	7.7	0.0383	-1.8	128.4	-11

N/A- Not Applicable

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Table 5: Statistical endpoint values.

Statistical Endpoint	Cell Density	Biomass (Area under the Growth Curve)	Growth Rate
NOAEC or EC ₀₅ (mg ai/L)	120	120	120
LOAEC	>120	>120	>120
IC ₅₀ or EC ₅₀ (mg ai/L) (95% C.I.)	>120 (N/A)	>120 (N/A)	>120 (N/A)
Other (EC ₁₀ and EC ₉₀)	N/A	N/A	N/A
Reference chemical, if used IC ₅₀ /EC ₅₀	N/A	N/A	N/A

ND – not determined

B. REPORTED STATISTICS:

Statistical analysis was performed for the endpoints of cell density, biomass (area under the growth curve) and growth rate. The data were tested for normality using Shapiro-Wilk's test ($\alpha = 0.01$), and for homogeneity of variance using Levene's test ($\alpha = 0.01$). Since the data demonstrated normality and homogeneity of variance, the treatment groups were compared to the control using ANOVA and Dunnett's test ($\alpha = 0.05$). The results of the statistical analyses and an evaluation of the concentration-response pattern were used to determine the NOAEC and LOAEC relative to each parameter at 96 hours. The EC₅₀ values could not be determined due to inhibitions for all endpoints being less than 50%. The study authors used SAS version 8.2 for statistical analyses.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Only replicate data for cell density were statistically-assessed to determine toxicity values, as inhibitions were <5% for biomass and growth rate. The reviewer visually determined toxicity values for those endpoints. The reviewer tested the replicate cell density data for normality using the Chi-square and Shapiro Wilk's tests and for homogeneity of variance using Hartley and Bartlett's test. If the assumptions of ANOVA were met, the NOAEC value was determined using the parametric Dunnett's and William's Test. If the assumptions were not met, the NOAEC value was determined using the non-parametric Steels or Kruskal-Wallis Test. The NOAEC value was determined using Toxstat Statistical Software. ECx values (with 95% C.I.) and probit slopes were determined using probit analyses via Nuthatch Statistical Software. All toxicity values were determined using the 96-hour mean-measured concentrations verified by the reviewer.

Cell density values were entered into Toxstat as the value $\times 10^4$.

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Cell Density

EC ₀₅ :	>120 mg ai/L	95% C.I.: N/A
EC ₅₀ :	>120 mg ai/L	95% C.I.: N/A
NOAEC:	120 mg ai/L	
Probit Slope:	N/A	

Biomass (Area Under the Growth Curve)

EC ₀₅ :	>120 mg ai/L	95% C.I.: N/A
EC ₅₀ :	>120 mg ai/L	95% C.I.: N/A
NOAEC:	120 mg ai/L	
Probit Slope:	N/A	

Growth Rate

EC ₀₅ :	>120 mg ai/L	95% C.I.: N/A
EC ₅₀ :	>120 mg ai/L	95% C.I.: N/A
NOAEC:	120 mg ai/L	
Probit Slope:	N/A	

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's results were identical to those of the study authors.

The reviewer verified the mean-measured concentrations and calculated the percent of nominal concentrations at 0 and 96 hours. The reviewer reports these unrounded values in the tables and statistical verification sections.

The study authors intended to determine if the test substance had an algistatic or algicidal effect, but inhibitions were so low that this determination was unnecessary.

Total organic carbon, particulate matter, and chlorine concentrations of the well water used for the algal medium were not reported.

The in-life portion of the algal toxicity test was conducted between December 3 and 7, 2007.

F. CONCLUSIONS:

This study is scientifically sound and classified as acceptable. The most sensitive endpoint could not be determined due to extremely low inhibitions at every test level for every endpoint evaluated, resulting in an overall NOAEC and EC₅₀ value of 120 and >120 mg ai/L, respectively.

Test Organism: *Skeletonema costatum*

Test Type (Flow-through, Static, Static Renewal): Static

Cell Density

EC ₀₅ :	>120 mg ai/L	95% C.I.: N/A
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EC₅₀: >120 mg ai/L 95% C.I.: N/A
NOAEC: 120 mg ai/L
Probit Slope: N/A

Biomass (Area Under the Growth Curve)

EC₀₅: >120 mg ai/L 95% C.I.: N/A
EC₅₀: >120 mg ai/L 95% C.I.: N/A
NOAEC: 120 mg ai/L
Probit Slope: N/A

Growth Rate

EC₀₅: >120 mg ai/L 95% C.I.: N/A
EC₅₀: >120 mg ai/L 95% C.I.: N/A
NOAEC: 120 mg ai/L
Probit Slope: N/A

Endpoint(s) Effected: None

III. REFERENCES:

Organization for Economic Cooperation and Development. 2006. OECD Guidelines for Testing of Chemicals, 201: *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*. Adopted 23 March 2006.

U.S. Environmental Protection Agency. 1996. Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II*.

ASTM Standard Guide 1218-90E. 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. American Society for Testing and Materials. Philadelphia, PA.

SAS Institute, Inc. 1999. SAS/STAT User's Guide, Version 8.2. Cary, NC, SAS Institute, Inc.

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

Aminocyclopyrachlor acid & 96-hr cell density; mg/L

File: 0202c Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.206	4.356	6.876	4.356	1.206
OBSERVED	0	6	6	6	0

Calculated Chi-Square goodness of fit test statistic = 3.7645

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Aminocyclopyrachlor acid & 96-hr cell density; mg/L

File: 0202c Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 12096.460

W = 0.943

Critical W (P = 0.05) (n = 18) = 0.897

Critical W (P = 0.01) (n = 18) = 0.858

Data PASS normality test at P=0.01 level. Continue analysis.

Aminocyclopyrachlor acid & 96-hr cell density; mg/L

File: 0202c Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 21.16

Closest, conservative, Table H statistic = 1362.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 2

Actual values ==> R (# groups) = 6, df (# avg reps-1) = 2.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test. (average df are used).

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Aminocyclopyrachlor acid & 96-hr cell density; mg/L
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Bartlett's test for homogeneity of variance

Calculated B statistic = 6.10
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 2.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

Aminocyclopyrachlor acid & 96-hr cell density; mg/L
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2063.509	412.702	0.409
Within (Error)	12	12096.460	1008.038	
Total	17	14159.969		

Critical F value = 3.11 (0.05,5,12)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Aminocyclopyrachlor acid & 96-hr cell density; mg/L
File: 0202c Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Neg control	287.233	287.233		
2	7.85	282.100	282.100	0.198	
3	15.5	283.700	283.700	0.136	
4	28.9	276.033	276.033	0.432	
5	60.0	271.467	271.467	0.608	
6	120	305.300	305.300	-0.697	

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Data Evaluation Report on the Acute Toxicity of Aminocyclopyrachlor acid technical to Algae (*Skeletonema costatum*)

PMRA Submission Number {.....}

EPA MRID Number 47560202

Aminocyclopyrachlor acid & 96-hr cell density; mg/L
File: 0202c Transform: NO TRANSFORMATION

DUNNETTS TEST		TABLE 2 OF 2		Ho:Control<Treatment		
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL	
1	Neg control	3				
2	7.85	3	64.809	22.6		5.133
3	15.5	3	64.809	22.6		3.533
4	28.9	3	64.809	22.6		11.200
5	60.0	3	64.809	22.6		15.767
6	120	3	64.809	22.6		-18.067

Aminocyclopyrachlor acid & 96-hr cell density; mg/L
File: 0202c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 1 OF 2			
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Neg control	3	287.233	287.233	280.107
2	7.85	3	282.100	282.100	280.107
3	15.5	3	283.700	283.700	280.107
4	28.9	3	276.033	276.033	280.107
5	60.0	3	271.467	271.467	280.107
6	120	3	305.300	305.300	305.300

Aminocyclopyrachlor acid & 96-hr cell density; mg/L
File: 0202c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)		TABLE 2 OF 2			
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Neg control	280.107				
7.85	280.107	0.275		1.78	k= 1, v=12
15.5	280.107	0.275		1.87	k= 2, v=12
28.9	280.107	0.275		1.90	k= 3, v=12
60.0	280.107	0.275		1.92	k= 4, v=12
120	305.300	0.697		1.93	k= 5, v=12

s = 31.750

Note: df used for table values are approximate when v > 20.